

CONTRAST-ENHANCED ULTRASOUND MONITORING OF PERFUSION CHANGES IN HEPATIC NEUROENDOCRINE METASTASES AFTER SYSTEMIC VERSUS SELECTIVE ARTERIAL $^{177}\text{Lu}/^{90}\text{Y}$ -DOTATOC AND ^{213}Bi -DOTATOC RADIOPEPTIDE THERAPY

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Aim: Radiopeptide therapy with beta emitter labeled $^{177}\text{Lu}/^{90}\text{Y}$ -DOTA(0)-Phe(1)-Tyr(3)-octreotide (DOTATOC) and more recently also alpha emitting ^{213}Bi -DOTATOC are promising new treatments for neuroendocrine tumors. No early predictors for treatment response have been recognized and tumor-shrinkage after radiation therapy appears slowly. In some solid tumors a decline in tumor perfusion was found predictive of final treatment response but the gold standard multiphase computed tomography (CT) has a high radiation burden. Therefore we evaluated the ability of contrast-enhanced ultrasound (CEUS) to evaluate tumor perfusion as a response criteria. **Materials and Methods:** 14 patients with hepatic neuroendocrine tumor (NET) metastases were enrolled in the retrospective study. Eleven patients were treated with beta-emitting $^{177}\text{Lu}/^{90}\text{Y}$ -DOTATOC, either intravenous (*i.v.*) ($n = 5$) or intra-arterial (*i.a.*) ($n = 6$) and three patients received alpha-emitting ^{213}Bi -DOTATOC (*i.a.*). CEUS and contrast-enhanced CT (CE-CT) were performed before and 3 months after treatment. **Results:** CE-CT and CEUS presented comparable results in the baseline study and in the assessment of perfusion changes due to the different treatment regimes. A therapy related decrease in tumor perfusion is an early predictor of longterm morphologic response. **Conclusion:** CEUS is available and radiation free technique which showed comparable results for perfusion and diameter of liver metastases compared to CE-CT. Intensity reduction in an arterial phase CEUS can be seen as a positive sign indicating long term tumor response to treatment. Therefore CEUS may be considered as an imaging modality for monitoring early treatment after focal alpha and beta targeted therapy.

Key Words: contrast-enhanced ultrasound, radionuclide therapy, treatment response, DOTATOC PET/CT.

Liver metastases are often the life-limiting factors for patients with gastroenteropancreatic neuroendocrine tumors (GEP-NET). Most GEP-NETs, as well as their liver

metastases are highly vascularised with a dense intramural vascular network [1]. Hence they show the typical peripheral contrast enhancement in the arterial phase of contrast-enhanced computed tomography (CE-CT) and contrast-enhanced ultrasound (CEUS). Moreover, they demonstrate an increased somatostatin receptor expression both in primary and metastatic lesions.

Concerning therapeutic and diagnostic options, the PET tracer DOTA(0)-Phe(1)-Tyr(3)-octreotide (DOTATOC), a ^{68}Ga -labelled somatostatin analog, has been shown to have high sensitivity and specificity for GEP-NET detection and staging [2]. Treatment with ^{90}Y - or ^{177}Lu -DOTATOC (beta emitters) is emerging as a potent therapy in patients with GEP-NETs. Systemic (*i.v.*) treatment can induce partial remission in 25–30% of the patients [3]. Tumor uptake of DOTATOC can be enhanced by loco-regional (*i.a.*) administration [4]; however, only patients with limited tumor extent are eligible for this approach. In comparison to beta emitters, alpha emitters have a higher linear energy transfer and potentially can induce tumor necrosis by structural damage of the targeted cell rather than radiation-induced apoptosis as occurred with beta irradiation.

Monitoring structural and functional characterisation of a tumor during therapy is important to tailor individual

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Abbreviations used: AASLD – American Association for the Study of Liver metastases; Bi – Bismut; cc – cubic centimetre; CE-CT – contrast-enhanced computed tomography; CEUS – contrast-enhanced ultrasound; CT – computed tomography; DOTATOC – $^{177}\text{Lu}/^{90}\text{Y}$ -DOTA(0)-Phe(1)-Tyr(3)-octreotide; DOTATOC PET – DOTATOC positron emission tomography; EANM – European Association of Nuclear Medicine; EASL – European Association for the study of the liver; Fig – Figure; Ga – Gallium; GBq – Gigabecquerel; GEP-NET – gastroenteropancreatic neuroendocrine tumors; HU – Hounsfield units; *i.a.* – intra-arterial; *i.v.* – intravenous; IRB – institutional review board; Lu – Lute-tium; MBq – Megabecquerel; Met – metastasis; MI – mechanical index; mRECIST – modified Response Evaluation Criteria in Solid Tumors; MRI – magnetic resonance imaging; *n* – number; NET – neuroendocrine tumor; Norm – normal liver parenchyma; OSEM – ordered subset expectation maximisation; PET – positron emission tomography; PET/CT – positron emission tomography/computed tomography; Phe – Phenylalanine; PRRT – peptide guided radioreceptor therapy; ROI – regions of interest; SNM – Society of Nuclear Medicine; Tyr – Tyrosine; Y – Yttrium.

patient care and evaluate novel treatment approaches. Since shrinkage of liver metastases — even with effective therapy — is rarely observed, modified response criteria, based on the degree of contrast enhancement, have been suggested by the American Association for the Study of Liver diseases (AASLD) and the European Association for the study of the liver (EASL) (modified Response Evaluation Criteria in Solid Tumors (mRECIST)). However, multi-phase CE-CT is associated with high radiation exposure. We investigated the role of CEUS to detect changes in perfusion as a non-invasive indicator of therapy response.

MATERIALS AND METHODS

Study design. 14 patients with hepatic NET metastases that have been treated with beta-emitting $^{177}\text{Lu}/^{90}\text{Y}$ -DOTATOC, either *i.v.* ($n = 5$) or *i.a.* ($n = 6$) or alpha-emitting ^{213}Bi -DOTATOC *i.a.* ($n = 3$).

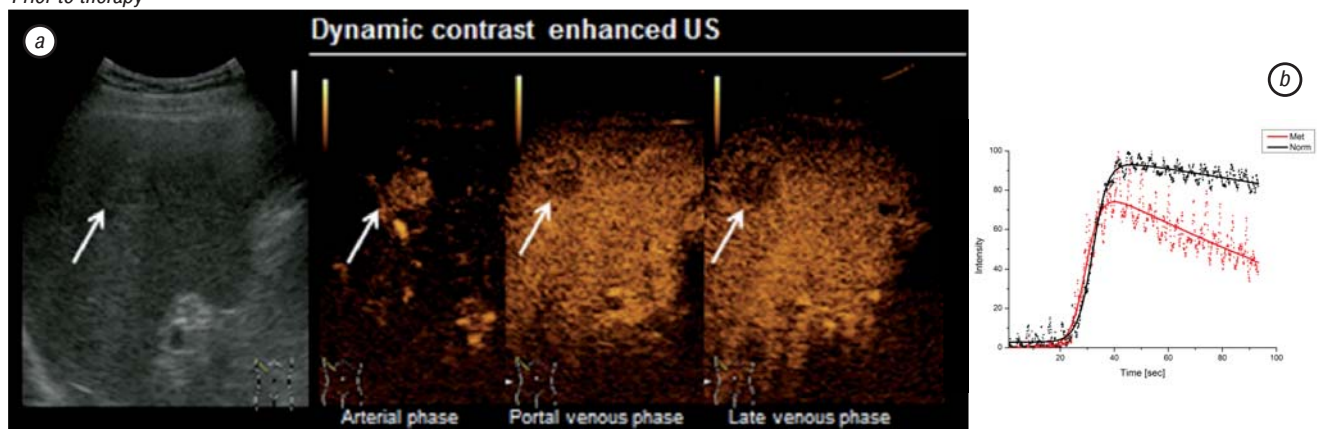
According to the Society of Nuclear Medicine (SNM) and European Association of Nuclear Medicine (EANM) guideline all patients prior to peptide guided radioreceptor therapy (PRRT) underwent a Somatostatin receptor (subtype 2 and 5) imaging procedure (^{68}Ga -DOTATOC or Octreotid-Scan) to elaborate the receptor expression. Patients who underwent surgery of the primary tumor (for example pancreatic cancer) and afterwards predominantly had their tumor load in the liver underwent loco-regional therapeutic treatment regime (*i.a.* DOTATOC-

Therapy). In contrast, patients who presented at the first imaging time point (^{68}Ga -DOTATOC or Octreotid-Scan) with an extensive systemic tumor spread underwent the systemic PRRT approach (*i.v.* DOTATOC-Therapy).

CEUS and CE-CT were performed as part of our clinical standard procedures and within the approved indications before and 3 months after treatment. A retrospective analysis was done and was approved by the institutional review board (IRB). Written informed consent was obtained from all patients. No prospective experiments with human subjects were performed and the study was in accordance with the Helsinki Declaration and our national regulations.

Contrast-enhanced ultrasound (CEUS). Prior to and 3 months after therapy, low-MI CEUS (FR = 19 fps, mechanical index MI = 0.15; HI VERSION Preirus, Hitachi, Switzerland) was performed. The examination was carried out with an intermittent breath-holding technique. One target lesion was identified in each patient by conventional ultrasound. Subsequently, dynamic imaging of these metastases was performed immediately after *i.v.* application of 2.4 cc of the contrast agent SonoVue® (Bracco S.P.A., Italy) with a duration of two minutes to monitor early arterial contrast enhancement and late portal-venous phase. The typical radiological feature of a liver metastasis of GEP-NETs was arterial phase hypervascularisation followed by portal-venous wash-out. Using a self-developed analysis software, regions

Prior to therapy



After therapy

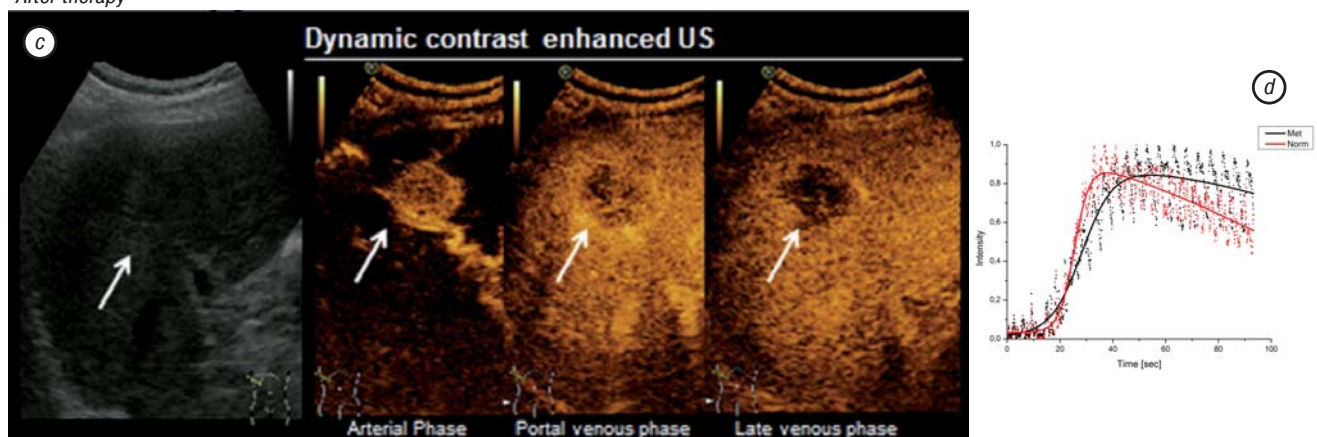
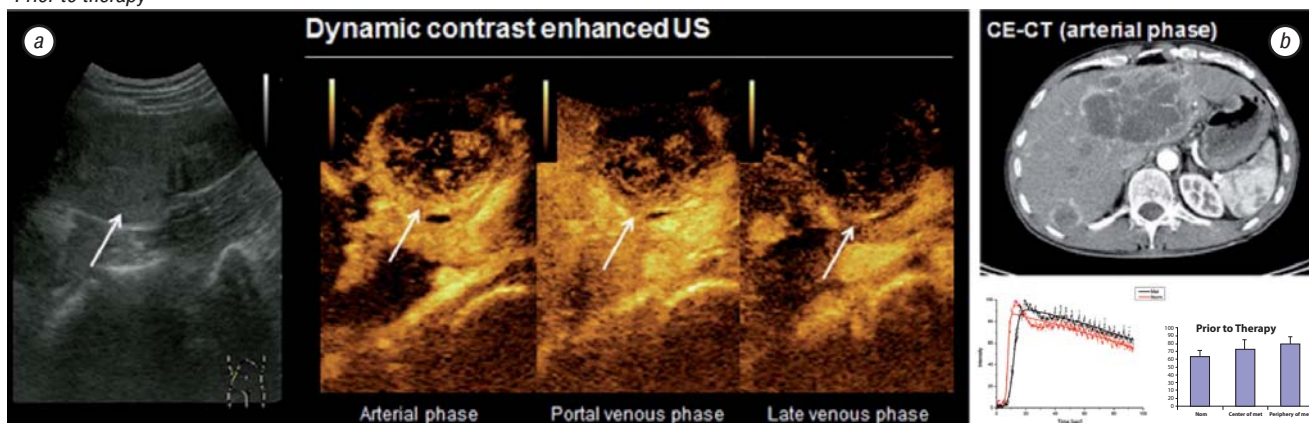


Fig. 1. Systemic therapy with 4+4 GBq ^{90}Y -/ ^{177}Lu -DOTATOC. a, b. CEUS image (a) and corresponding time-intensity curve (b) of a neuroendocrine metastasis and normal liver parenchyma before therapy. c, d. CEUS image (c) and corresponding time-intensity curve (d) of a neuroendocrine metastasis and normal liver parenchyma 3 months after systemic therapy with 4+4 GBq ^{90}Y -/ ^{177}Lu -DOTATOC. White arrows indicate metastatic lesion. Undulations in curves are caused by breathing artefacts. An increased uptake in the arterial phase after therapy (d) was associated with tumor progression in the follow-up. Met — metastasis; Norm — Normal liver parenchyma

Prior to therapy



After therapy

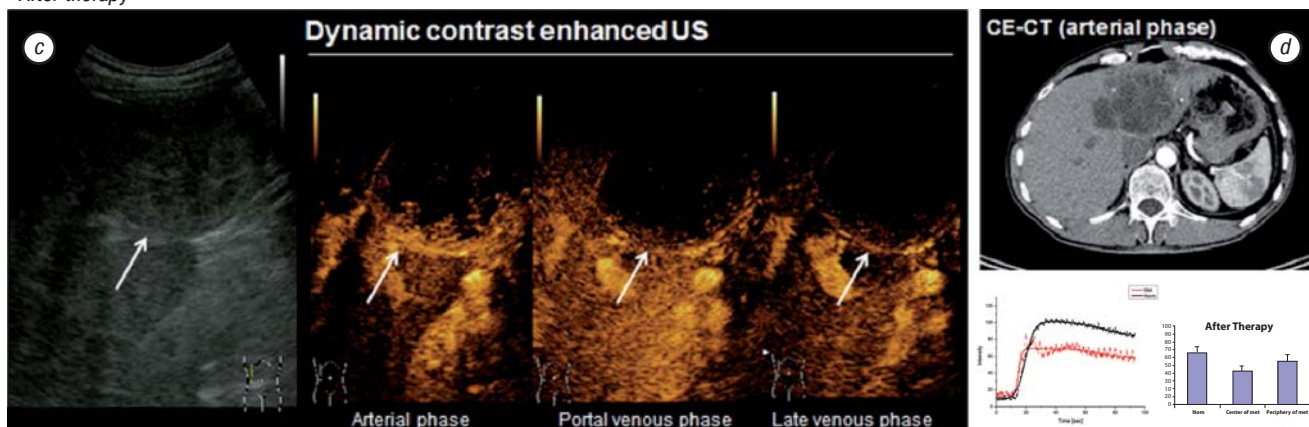


Fig. 2. Intra-arterial therapy with 4+4 GBq $^{90}\text{Y-}/^{177}\text{Lu}$ -DOTATOC. *a, b.* CEUS (*a*) and corresponding time-intensity curves (*b*) of a neuroendocrine metastasis and normal liver parenchyma prior to therapy. *c, d.* CEUS (*c*) and corresponding time-intensity curves (*d*) of a neuroendocrine metastasis and normal liver parenchyma 3 months after i.a. therapy with 4+4 GBq $^{90}\text{Y-}/^{177}\text{Lu}$ -DOTATOC. White arrows indicate metastatic lesion. A decline in tumor contrast enhancement was observed in CEUS (*a, c*) and equal to multi-phase CT (*b, d*) and was associated with tumor shrinkage in the long term follow-up. Met — metastasis; Norm — Normal liver parenchyma.

of interest (ROI) were marked in the liver metastases and in adjacent normal liver parenchyma, measuring change in intensity over time. Peak image intensity and time to peak were recorded for each ROI.

Contrast-enhanced computed tomography (CE-CT). Prior to and 3 months after therapy, contrast-enhanced CT was performed in each patient using a Siemens Biograph 6 Positron Emission Tomography/Computed

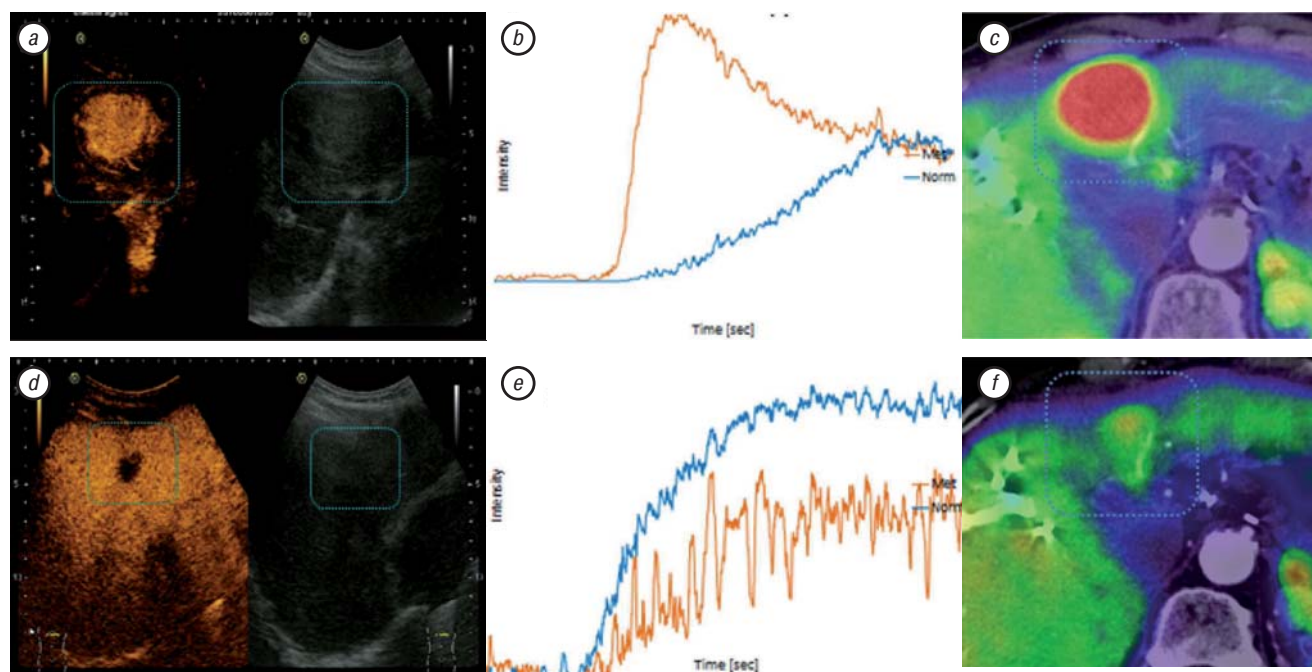


Fig. 3. Intra-arterial therapy with 4+2 GBq $^{90}\text{Y-}/^{177}\text{Lu}$ DOTATOC. *a, b.* CEUS (*a*) and corresponding time-intensity curves (*b*) of a neuroendocrine metastasis and normal liver parenchyma before therapy. *d, e.* CEUS (*d*) and corresponding time-intensity curves (*e*) of a neuroendocrine metastasis and normal liver parenchyma 3 months after i.a. therapy. *c, f.* Sequential hybrid imaging of ^{68}Ga -DOTATOC PET/CT before (*c*) and after (*f*) i.a. therapy also confirm CEUS finding. Met — metastasis; Norm — Normal liver parenchyma

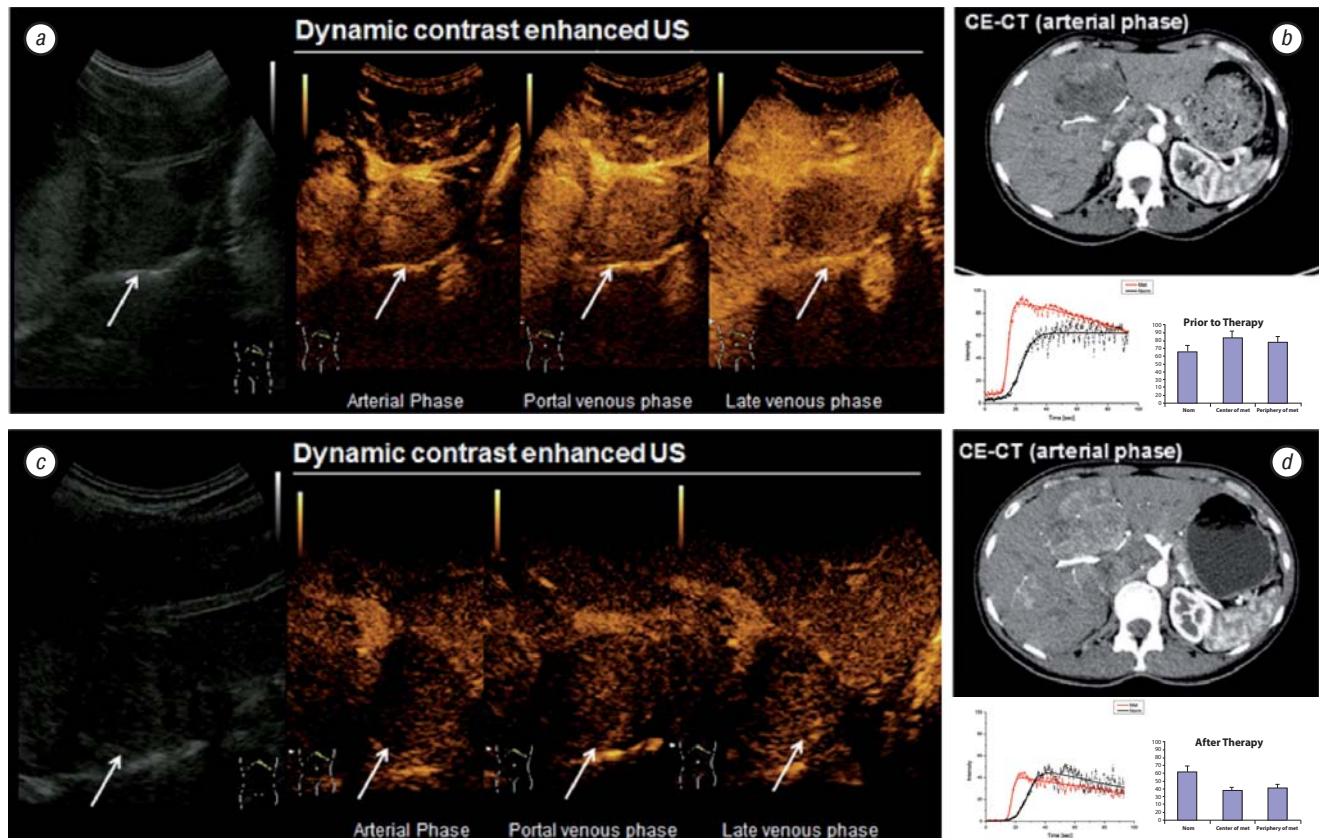


Fig. 4. Intra-arterial therapy with ^{213}Bi -DOTATOC. *a, b.* CEUS (*a*) and corresponding time-intensity curves (*b*) of a neuroendocrine metastasis and normal liver parenchyma before therapy. *c, d.* CEUS (*c*) and corresponding time-intensity curves (*d*) of a neuroendocrine metastasis and normal liver parenchyma 3 months after i.a. therapy with ^{213}Bi -DOTATOC. White arrows indicate metastatic lesion. Positive contrast in arterial and portal venous phase decline after therapy — this was similar in both CEUS and CT. Met — metastasis; Norm — Normal liver parenchyma

Tomography (PET/CT). A ROI of 40 pixels was marked in the center and outer rim of each evaluated metastatic lesion in the arterial phase of the CE-CT. The ratio of intensity of lesions and normal liver parenchyma was recorded measuring corresponding Hounsfield units (HU).

DOTATOC positron emission tomography (DOTATOC PET). DOTATOC-PET/CT was also performed on the Biograph 6 (Siemens, Knoxville, USA). Imaging was initiated about 45 min after i.v. injection of 89–189 MBq ^{68}Ga -DOTATOC. Static emission scans, corrected for dead time, scatter and decay were acquired from the vertex to the proximal legs — requiring eight bed positions, 4 min each. The low-dose CT without contrast agent was used for attenuation correction. The images were iteratively reconstructed with the OSEM (ordered subset expectation maximisation) algorithm using four iterations with eight subsets and Gaussian filtering to achieve an in-plane spatial resolution of 5 mm at full-width half-maximum.

RESULTS

Prior to therapeutic intervention, all liver metastases demonstrated marked enhancement on both CE-CT and CEUS in all three treatment groups.

For the *i.v.* group (beta radiation) a significant decline of enhancement was observed in 1/5 patients 3 months after intervention. In 3/5 patients no change in enhancement was seen and in one patient (Fig. 1) the lesion progressed.

For the *i.a.* group (beta radiation) 3/6 patients demonstrated both a decline of tumor vascularity

as assessed with CEUS and of diameter as assessed with arterial phase CT (Fig. 2) and also a decline in SSR2 expression in DOTATOC PET imaging (Fig. 3).

For the *i.a.* group, treated with ^{213}Bi -DOTATOC (alpha radiation), CEUS and arterial phase CT demonstrated a decrease of tumor perfusion (Fig. 4).

Regarding contrast intensity in CEUS in the *i.a.* group treated with ^{213}Bi -DOTATOC (relative values), metastases had a 40% higher contrast intensity than normal liver parenchyma with an early onset of contrast enhancement before treatment (metastatic liver parenchyma set as 100% with an early onset of contrast enhancement; intensity in normal liver parenchyma was measured 60% of metastatic liver parenchyma (Fig. 4 *b*)) to almost equilibrium 3 months after therapy (normal liver parenchyma and metastases reached the same level of intensity while metastases still showed an earlier contrast enhancement than normal liver parenchyma (Fig. 4 *d*)). In CE-CT (absolute values), measuring Hounsfield units in peripheral regions of metastases, *i.a.* application of ^{213}Bi -DOTATOC led to a 50% decrease of density values within 3 months (800 HU before treatment, 400 HU 3 months later (Fig. 4 *b, d*)). Simultaneously, *i.a.* application of ^{213}Bi -DOTATOC did not lead to a significant shrinkage of metastases within 3 months. Nevertheless, in the longterm follow up (9 months) none of these lesions progressed.

The absence of intensity reduction in an arterial phase CEUS can be seen as a negative sign indicating unresponsiveness to treatment.

DISCUSSION

As described above, patients with systemic tumor manifestations outside the liver underwent the systemic PRRT approach (i.v. DOTATOC-Therapy). Patients without tumor manifestations outside the liver underwent the loco-regional therapeutic treatment regime (i.a. DOTATOC-Therapy), irrespective of the size and number of liver metastases, according to the SNM- and EANM guidelines.

In patients with liver-metastases from GEP-NETs i.a. treatment with $^{177}\text{Lu}/^{90}\text{Y}$ -DOTATOC lead to a significant decrease of tumor vascularity and diameter, compared to treatment with systemic treatment. The observed response seen with targeted beta radiation administered either via i.v. or i.a. was in accordance with prior results. A decline in lesion perfusion correlated with tumor shrinkage on arterial phase CT [5]. In contrast, high energy alpha radiation produced marked reduction in tumor vascularity with little or no change in tumor diameter after several months. Thus, this data suggests that alpha irradiation leads to early changes in tumor microcirculation, occurring prior to tumor shrinkage.

Comparing CEUS and CE-CT, results of tumor perfusion and diameter were equal in both modalities, regardless of the therapy regimen. One important point to mention is that the first structural changes after PRRT are visible about six weeks after therapy in CEUS and CE-CT. However, morphological changes, which can be detected with CT and CEUS need at least a follow up period of 6 months to become visible. This coincides with findings from evaluations of unclear liver lesions, where contrast enhanced ultrasound was even more accurate than multisliced computed tomography in predicting malignancy and benignity [6]. This applies especially to lesions in the caudal and ventral parts of the liver, which can easily be visualized by ultrasound. Problems in the evaluation of subdiaphragmatic lesions are still unchanged, which is a limitation for the use of ultrasound for follow-up examinations in these liver segments. Under optimal ultrasound conditions, when the whole liver can be imaged, CEUS reaches a diagnostic certainty of > 90% and is as reliable as contrast-enhanced MRI, and beats CE-CT [7, 8]. Correspondingly high sensitivity rates of CEUS in liver tumors have been reported in many recent works [9].

A limiting factor of this study is the relatively small number of 14 patients that has been examined. That was due to the fact that not all of the patients with liver metastases of GEP-NETs in our hospital were available

for a comparison of CEUS, CE-CT and DOTATOC-PET/CT. In each patient we examined no more than one target lesion due to technical requirements in CEUS.

In summary CEUS is a reliable method to monitor early changes in metastases even before true volume shrinkage occurs. After focal alpha targeted therapy results in CEUS were as valuable as in CE-CT, which is the common way of examining tumor response. CEUS is typically available at lower cost and without additional ionizing radiation compared to CT, and it is portable. It increases individual patient care and reduces costs. Therefore, CEUS may be considered as an additional imaging modality to assess the early microvascular tumor environment in follow-up studies after focal alpha targeted therapy.

CONFLICT OF INTEREST DISCLOSURE

The authors declared no conflicts of interest.

REFERENCES

1. Villaume K, Blanc M, Gouysse G, *et al.* VEGF secretion by neuroendocrine tumor cells is inhibited by octreotide and by inhibitors of the PI3K/AKT/mTOR pathway. *Neuroendocrinology* 2010; **91**: 268–78.
2. Ruf J, Heuck F, Schiefer J, *et al.* Impact of Multiphase ^{68}Ga -DOTATOC-PET/CT on therapy management in patients with neuroendocrine tumors. *Neuroendocrinology* 2010; **91**: 101–9.
3. Kwekkeboom DJ, de Herder WW, Kam BL, *et al.* Treatment with the radiolabeled somatostatin analog [^{177}Lu -DOTA 0, Tyr3]octreotate: toxicity, efficacy, and survival. *J Clin Oncol* 2008; **26**: 2124–30.
4. Forner A, Ayuso C, Varela M, *et al.* Evaluation of tumor response after locoregional therapies in hepatocellular carcinoma: are response evaluation criteria in solid tumors reliable? *Cancer* 2009; **115**: 616–23.
5. Kratochwil C, Lopez-Benitez R, Mier W, *et al.* Hepatic arterial infusion enhances DOTATOC radiopeptide therapy in patients with neuroendocrine liver metastases. *Endocr Relat Cancer* 2011; **18**: 595–602.
6. Clevert DA, Jung EM, Stock KF, *et al.* Evaluation of malignant liver tumors: biphasic MS-CT *versus* quantitative contrast harmonic imaging ultrasound. *Z Gastroenterol* 2009; **47**: 1195–202.
7. Boozari B, Lotz J, Galanski M, *et al.* Diagnostic imaging of liver tumors. Current status. *Internist (Berl)* 2007; **48**: 8–20.
8. Giesel FL, Kratochwil C, Mehndiratta A, *et al.* Comparison of neuroendocrine tumor detection and characterization using DOTATOC-PET in correlation with contrast enhanced CT and delayed contrast enhanced MRI. *Eur J Radiol* 2012; **81**: 2820–5.
9. Celli N, Gaiani S, Piscaglia F, *et al.* Characterization of liver lesions by real-time contrast-enhanced ultrasonography. *Eur J Gastroenterol Hepatol* 2007; **19**: 3–14.